

**Data Evaluation Record on the Acute Oral Toxicity of Flufenacet Technical to Canary
(*Serinus canaria*)**

PMRA Submission Number {.....}

EPA MRID Number 49244202

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	416448
	OECD Data Point	{.....}
	EPA MRID	49244202
	EPA Guideline	850.2100

Test material: Flufenacet Technical **Purity:** 98.83%
Common name Flufenacet
Chemical name: IUPAC: 4'-fluoro-*N*-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide
CAS: *N*-(4-fluorophenyl)-*N*-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide
CAS No.: 142459-58-3
Synonyms: FOE5043 Technical; AE F133402

Primary Reviewer: Christie E. Padova
Staff Scientist, CSS-Dynamac Corporation

Signature: 
Date: 02/19/14

Secondary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM Smith

Signature: 
Date: 9/25/14

Primary Reviewer: Geoffrey Sinclair, Biologist
{EPA/OECD/PMRA}

Date: 11/20/14

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	121903	

Date Evaluation Completed: dd-mm-yyyy

CITATION: Christ, M.T. 2013. Toxicity of Flufenacet Technical During an Acute Oral LD₅₀ with the Canary (*Serinus canaria*). Unpublished study performed by SynTech Research Laboratory Services, LLC, Stilwell, KS. Laboratory Project ID: 07SRLS13C5. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated September 9, 2013 and completed October 25, 2013.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute oral toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

The acute oral toxicity of flufenacet to adult canaries (*Serinus canaria*) was assessed over 14 days. Flufenacet technical was administered neat to the birds via corn oil-coated gelatin capsules at nominal doses of 0, 135, 236, 413, 723 and 1265 mg ai/kg bw (adjusted for purity). The 14-day acute oral LD₅₀ (with 95% C.I.) was 461 (291 to 598) mg ai/kg bw. According to the U.S. EPA classification system, flufenacet would be classified as moderately toxic to adult canary on an acute oral basis.

Cumulative mortality was 10, 0, 10, 40, 90 and 100% in the control, 135, 236, 413, 723 and 1265 mg ai/kg bw dose levels, respectively; all treatment-related deaths occurred by Day 1, with no gender-related differences. Ataxia and diminished reaction to stimuli (hypo-reactivity) were observed in birds from the ≥ 236 mg ai/kg dose levels. Effects were noted within 1 hour of dosing, and surviving birds from all levels recovered by Day 2.

No remarkable findings were seen upon necropsy of birds that died during the study. There was a suggestive decrease in male body weight gain at all treatment levels relative to the negative control, whereas decreases were apparent for females in the 236, 413, and 723 mg ai/kg bw treatment groups. Food consumption appeared comparable between the control and treatment groups.

This study is scientifically sound and fulfills the guideline requirements for an acute oral toxicity study with a passerine species. It is therefore classified as acceptable.

Results Synopsis

Test Organism Size/Age (Mean Weight): Mixed adult (3 months to 3 years old); 18.3 to 24.4 g (mean of 21.6 \pm 1.5 g, combined sexes)

LD ₅₀ : 461 mg ai/kg bw	95% C.I.: 291-598 mg ai/kg bw
Slope: 6.44	95% C.I.: 2.00-10.9

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I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: The study protocol was based upon methods outlined in the U.S. EPA Ecological Effects Test Guidelines, OPPTS No. 850.2100 (1996).

No notable deviations from OCSPP 850.2100 guidance (2012) were observed.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP standards as published by the U.S. EPA (40 CFR Part 160) with the following exceptions: genetic analysis to determine the sex of the birds, and contaminant screening analysis of the bird feed, tap water, and corn oil (used to coat the capsule prior to administration). In addition, the test material expired prior to experimental start.

A. MATERIALS:

1. Test material Flufenacet technical

Description: Solid, light beige color

Lot No./Batch No. : AE F133402-01-19

Purity: 98.83%

Stability of compound under test conditions: N/A; the test substance was administered neat

Storage conditions of test chemicals: Ambient temperature

Physicochemical properties of flufenacet.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test Organism:

Species (common and scientific names): Canary (*Serinus canaria*)

Age at study initiation: Adult; females – 3 months to 3 years old, males – 4 months to 2 years old

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Weight at study initiation (mean and range): 18.3 to 24.4 g (mean of 21.6 ± 1.5 g) (combined sexes)

Source: Maryland Exotic Birds, Pasadena, MD

(EPA recommends using either bobwhite quail or mallard duck. Birds should be at least 16 weeks old at test initiation and should be uniform in size and weight as well as phenotypically indistinguishable from wild birds).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Several (not otherwise specified) non-GLP range-finding studies were conducted. In one trial, two birds for the 31 mg/kg level and three birds per level for the following doses were capsule-dosed at 31, 63, 125, 250, 500, 100 and 2000 mg ai/kg bw. Total mortality was observed at the ≥ 250 mg/kg levels, one bird died at the 125 mg/kg level, and no mortality was observed at the 31 or 63 mg/kg levels. Sub-lethal effects were observed at all dose levels; effects included lethargy and loss of muscle control.

An initial definitive-study attempt was conducted at nominal levels of 0 (control), 60, 90, 135, 202.5 and 304 mg/kg bw. However, following the 14-day study, only 10 and 20% mortality occurred at the 202.5 and 304 mg/kg bw levels, respectively. Based on these results, the study was repeated at higher dose levels.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Acclimation</u> Period:	ca. 2 weeks	Birds were individually-caged and all appeared normal during acclimation.
Conditions: (same as test or not)	Same as test	<i>The recommended acclimation period is a minimum of 15 days. OECD recommends a minimum of 7 days.</i>
Feeding:	Lab Diet Advanced Protocol Small Avian Maintenance (PMI Nutrition International, Brentwood, MO) and tap water from Kansas City, MO public water supply were available <i>ad libitum</i> .	
Health: (any mortality observed)	All birds were in good health upon arrival and only birds that appeared healthy were used for testing. Less than 1% mortality occurred in the population during acclimation.	

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Parameter	Details	Remarks
		<i>Criteria</i>
Pen size and construction materials	Cages were constructed of metal bars and sheeting and measured 27 (L) x 33 (W) x 31 m (H).	
		<i>Pen size and construction should conform to good husbandry practices and should not create crowding stress.</i> <i>OECD recommends that pens be suitable for the captive rearing of that species.</i>
Test duration	14 days	
		<i>Recommended test duration is one day for dosing and at least 14 days observation.</i>
Dose preparation [Indicate method of confirmation of dose]	The test substance was dosed neat.	
Mode of dose administration	Each bird was dosed with one Torpac® #9 gelatin capsule. Each capsule was coated with corn oil and inserted into the bird's crop.	Birds were weighed within 28 hours prior to dosing.
		<i>Gavage or gelatin capsule is recommended</i>
<u>Dose levels</u> nominal:	0 (carrier control), 135, 236, 413, 723 and 1265 mg ai/kg bw N/A	Male and female birds were randomly assigned to each treatment group using a computerized randomization program based on body weight.
		<i>Dose levels should be a minimum of 5 treatment levels unless LD₅₀ is demonstrated to be greater than 2000 mg ai/kg</i>
<u>Solvent/vehicle, if used</u> type: amount/bw:	N/A	
		<i>The test material should be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.</i>

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Parameter	Details	Remarks
		Criteria
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	10 (5 per sex) N/A 10 (5 per sex) per level	Recommended number of birds in a treatment group is 10 and 10 birds for each control and vehicle group.
No. of feed withholding days before dosing	≥4 hours	Food should be withheld for at least 15 hours prior to dosing.
Test conditions Temperature:	Daily average: 22.2°C	Light intensity averaged 192 lux.
Relative humidity:	Daily average: 51%	The recommended photoperiod is 10 hours of light and 14 hours of dark.
Photoperiod:	8 hours light:16 hours dark	
Reference chemical, if used name: concentrations tested:	None tested	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
		Criteria
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Body weight - Food consumption	Body weight should be measured at test initiation, on day 14 and at the end of the test if the test is extended beyond 14 days. Mortality should not be more than 10% in controls. Feed consumption should be measured as average daily food consumption.
Indicate if the test material was regurgitated	No regurgitation of the test substance was noted in any test group.	Regurgitation is an indication that the dose was rejected. If this problem persists, the test should be repeated.

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Criteria	Details	Remarks
		<i>Criteria</i>
Groups on which necropsies were performed	Birds that died during the course of the study were subjected to gross necropsy.	<i>Gross necropsies should be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.</i>
Observation intervals	<p>Mortality and signs of toxicity were observed three times on Day 0 (<i>ca.</i> 1, 2, and 3 hours post-dosing) and at least once daily thereafter.</p> <p>Body weights were measured individually on Days -1, 7, and 14.</p> <p>Feed consumption was recorded daily.</p>	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Cumulative mortality was 10, 0, 10, 40, 90 and 100% in the control, 135, 236, 413, 723 and 1265 mg ai/kg bw dose levels, respectively. All deaths occurred by Day 1, except for a single accidental control mortality on Day 14. No gender-related differences were indicated. The lowest lethal dose was 236 mg ai/kg bw. The 14-day LD₅₀ (with 95% C.I.) was 5.5 (4.0 to 7.5) mg ai/kg bw.

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Table 3: Effect of Flufenacet on Mortality of Canary.

Treatment (mg ai/kg bw)		No. of Birds	Cumulative Mortality				
			day 0	day 1	day 3	day 7	day 14
Control		10	0	0	0	0	1 (♀)
135		10	0	0	0	0	0
236		10	1 (♂)	1	1	1	1
413		10	2 (1♂, 1♀)	4 (1♂, 3♀)	4	4	4
723		10	7 (5♂, 2♀)	9	9	9	9
1265		10	5 (2♂, 3♀)	10	10	10	10
NOAEL		135 mg ai/kg bw					
LD ₅₀ (with 95% C.I.)		434 (337 to 560) mg ai/kg bw Probit slope: 5.6 (2.9 to 8.3)					
Reference chemical	mortality	N/A					
	LD ₅₀	N/A					
	NOAEL	N/A					

B. SUB-LETHAL TOXICITY ENDPOINTS:

All control birds and birds from the 135 mg/kg dose group appeared normal throughout the study, although one control bird was found dead in the water dish following observations on Day 14 (death was considered accidental). Clinical signs of toxicity, specifically diminished reaction to stimuli (hypo-reactivity) and/or ataxia were observed in birds from the ≥ 236 mg ai/kg dose levels. Effects were noted within 1 hour of dosing, and surviving birds from the 236 and 723 mg ai/kg bw dose levels recovered by Day 1, while surviving birds from the 413 mg ai/kg bw dose level recovered by Day 2.

No remarkable findings were seen upon necropsy of birds that died during the study.

No statistically-significant differences were observed on male or female body weights (Days -1, 7 and 14) or on male or female body weight changes (Days -1 to 7, 7 to 14, and -1 to 14) at any dose level compared to the control. When sexes were combined, no statistically-significant differences were observed on body weights at any interval or dose level, although there was a statistically-significant decrease in body weight gain at the 236 mg ai/kg bw level compared to the control for Days -1 to 14 ($\alpha=0.05$).

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Table 4: Effect of Flufenacet on Body Weight and Body Weight Changes (g \pm SD) of Canary.^(a)

Males						
Treatment (mg ai/kg bw)	Day -1	Day 7	Day 14	Days -1 to 7	Days 7 to 14	Days -1 to 14
Control	21.9 ± 1.6	22.2 ± 0.7	23.6 ± 0.8	0.3 ± 1.8	1.5 ± 0.3	1.7 ± 2.0
135	22.1 ± 1.7	21.7 ± 1.9	22.5 ± 1.8	-0.4 ± 0.8	0.8 ± 0.3	0.4 ± 0.6
236	22.1 ± 1.5	21.5 ± 2.2	22.9 ± 1.5	-0.9 ± 1.3	1.4 ± 0.9	0.4 ± 0.4
413	21.5 ± 1.6	20.4 ± 1.0	22.1 ± 1.0	-1.6 ± 0.6	1.8 ± 0.4	0.2 ± 0.8
723	21.6 ± 1.6	---	---	---	---	---
1265	21.8 ± 1.7	---	---	---	---	---
NOAEL	Not reported					
EC ₅₀	Not reported					
Females						
Treatment (mg ai/kg bw)	Day -1	Day 7	Day 14	Days -1 to 7	Days 7 to 14	Days -1 to 14
Control	21.4 ± 1.8	20.4 ± 2.3	22.2 ± 2.5	-1.0 ± 0.7	1.4 ± 0.1	0.6 ± 0.6
135	21.8 ± 1.8	20.8 ± 1.9	22.2 ± 2.0	-0.9 ± 0.4	1.4 ± 0.4	0.5 ± 0.3
236	21.7 ± 1.3	20.0 ± 0.9	20.9 ± 1.0	-1.7 ± 1.4	0.9 ± 0.6	-0.7 ± 1.3
413	20.9 ± 1.9	18.3 – 20.2	20.0 – 21.4	-2.1 – -1.3	1.2 – 1.7	-0.9 – 0.4
723	21.1 ± 1.9	18.2	19.5	-1.9	1.3	-0.6
1265	21.3 ± 1.8	---	---	---	---	---
NOAEL	Not reported					
EC ₅₀	Not reported					

^(a) Range reported when only two surviving birds present; value reported when only one surviving bird present.

No statistically-significant differences were observed on the food consumption of males or females at any interval or dose level.

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Table 5: Effect of Flufenacet on Food Consumption (g/bird/day) of Canary.^(a)

Males			
Treatment (mg ai/kg bw)	Days 1 to 7	Days 8 to 14	Days 1 to 14
Control	6.1 ± 0.8	5.9 ± 1.4	6.0 ± 1.1
135	5.5 ± 1.3	5.4 ± 0.8	5.4 ± 1.0
236	5.0 ± 0.8	5.1 ± 0.5	5.0 ± 0.5
413	5.0 ± 1.0	5.5 ± 1.3	5.3 ± 1.1
723	---	---	---
1265	---	---	---
NOAEL	Not reported		
EC ₅₀	Not reported		
Females			
Treatment (mg ai/kg bw)	Days 1 to 7	Days 8 to 14	Days 1 to 14
Control	5.2 ± 1.8	4.8 ± 1.1	5.0 ± 1.5
135	4.8 ± 0.6	4.6 ± 0.2	4.7 ± 0.3
236	5.9 ± 1.5	6.0 ± 2.4	6.0 ± 1.9
413	5.5 ± 1.1	4.9 ± 1.1	5.2 ± 1.1
723	4.6	3.9	4.3
1265	---	---	---
NOAEL	Not reported		
EC ₅₀	Not reported		

^(a) Range reported when only two surviving birds present; value reported when only one surviving bird present.

C. REPORTED STATISTICS:

The 14-day LD₅₀ (with 95% C.I.) was determined using Probit Analysis using CT-TOX software. Body weight, body weight changes and food consumption data were assessed for normality using the Chi-Square test ($\alpha = 0.01$) and for homogeneity of variance using Levene's test ($\alpha = 0.05$). All data were normally distributed and the variances were homogenous, so the data were subjected to ANOVA followed by a one-tailed Dunnett's test or a Bonferroni's-adjusted t-test ($\alpha = 0.05$). Analyses were performed using TOXSTAT statistical software.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 14-day LD₅₀, probit slope, and associated 95% confidence intervals were estimated using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented on 3/25/14. Sex-specific body weight change and associated standard errors were entered into CETIS but not analyzed.

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All analyses were conducted at $\alpha = 0.05$ using nominal concentrations.

LD ₅₀ : 461 mg ai/kg bw	95% C.I.: 291-598 mg ai/kg bw
Slope: 6.44	95% C.I.: 2.00-10.9

E. STUDY DEFICIENCIES:

There were no notable deviations or deficiencies affecting the scientific soundness or acceptability of this study.

F. REVIEWER'S COMMENTS:

The study author's LD₅₀ was slightly lower than the reviewer's. The reviewer's results are reported in the Executive Summary and Conclusions section of this DER.

Due to a shortage of male birds, birds from two lots were used: the first lot (from which 30 females were selected) arrived on June 10, 2013 and the second lot (from which 30 males were selected) arrived on August 12, 2013. It was reported that the range of body weights at study initiation was $\pm 15\%$ of the mean body weight due to the limited number of birds with an equal sex ratio.

The photoperiod (8 hr light:16 hr dark) was slightly less than recommended (10 hr light:14 hr dark).

As the test substance was administered neat in capsules, confirmation of dosage as well as stability of the test substance during the dosing procedure were not applicable.

Experimental study dates were September 10 to 24, 2013.

G. CONCLUSIONS:

This study is scientifically sound and is classified as acceptable. There was a suggestive decrease in male body weight gain at all treatment levels relative to the negative control, whereas decreases were apparent for females in the 236, 413, and 723 mg ai/kg bw treatment groups. Food consumption appeared comparable between the control and treatment groups. There were no abnormalities at necropsy of decedent birds. The 14-day LD₅₀ value was 461 mg ai/kg bw.

LD ₅₀ : 461 mg ai/kg bw	95% C.I.: 291-598 mg ai/kg bw
Slope: 6.44	95% C.I.: 2.00-10.9

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III. REFERENCES:

Haze, T. 1990. CT-TOX multi-method program, Version 1.1, Bureau of Water Management, Connecticut
Department of Environmental Protection, Water Toxics Laboratory, Hartford, CT 06106, USA.

West, Inc., and D.D. Gulley. 1994. TOXSTAT Version 3.4. West, Inc., Western EcoSystems Technology, Inc.,
Cheyenne, WY 82001, USA.

CETIS Summary Report

Report Date: 20 Apr-14 14:03 (p 1 of 1)
 Test Code: 121903 49244202 | 18-3847-3757

OCSPP 850.2100 Acute Avian Oral Toxicity				SynTech Research Laboratory Services LLC			
Batch ID:	15-1759-8033	Test Type:	Acute Avian Oral Toxicity	Analyst:			
Start Date:	10 Sep-13	Protocol:	OCSPP 850.2100 Acute Bird	Diluent:	Corn Oil		
Ending Date:		Species:	Serinus canaria	Brine:	Not Applicable		
Duration:	NA	Source:	Maryland Exotic Birds, Pasadena	Age:	<3yr		
Sample ID:	03-0956-1316	Code:	49244202	Client:	CDM Smith		
Sample Date:	10 Sep-13	Material:	Flufenacet	Project:	Herbicide		
Receive Date:		Source:	Bayer CropScience AG				
Sample Age:	NA	Station:					
Batch Note: PC Code 121903 MRID 49244202							
Sample Note: PC Code 121903 MRID 49244202							

Point Estimate Summary							
Analysis ID	Endpoint	Level	mg ai/kgB	95% LCL	95% UCL	TU	Method
00-7168-4931	14dMortalityRate	LC5	256	51.3	361		Linear Regression (MLE)
		LC10	292	77	394		
		LC15	319	101	420		
		LC20	342	125	443		
		LC25	363	149	465		
		LC40	422	230	536		
16-0349-1867	14dMortalityRate	LC50	461	291	598		Spearman-Kärber
		LC50	458	368	569		

14dMortalityRate Summary											
C-mg ai/kgB	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	1	0.1			0.1	0.1	0	0	0.0%	0.0%
135		1	0			0	0	0	0		-11.1%
236		1	0.1			0.1	0.1	0	0	0.0%	0.0%
413		1	0.4			0.4	0.4	0	0	0.0%	33.3%
723		1	0.9			0.9	0.9	0	0	0.0%	88.9%
1265		1	1			1	1	0	0	0.0%	100.0%

14dMortalityRate Detail		
C-mg ai/kgB	Control Type	Rep 1
0	Negative Control	0.1
135		0
236		0.1
413		0.4
723		0.9
1265		1